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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS	:	Miller, et al.
TITLE	:	POLYCATIONIC STEROL DERIVATIVES AS TRANSFECTION AGENTS
APPLICATION NO.	:	10/770,294
FILED	:	February 2, 2004
CONFIRMATION NO.	:	1414
EXAMINER	:	Ford, Vanessa L.
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DECLARATION OF FACT (UNDER 37 C.F.R. 1.132) OF MICHAEL KELLER, PhD

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

As the person signing below,

1. I, Dr. Michael Keller, do hereby declare that I am currently the Chief Scientist at IC-Vec Ltd., 13 Prince's Gardens, London, England, SW7 1NA, where my research focuses on developing targeted non-viral vectors for *in vivo* applications.
2. I have a degree in natural sciences from The Swiss Federal Institute of Technology, also known as ETH Zurich (Zurich, Switzerland); a Masters of Science in chemical research from Imperial College London; a DEA (Diplôme d'Etudes Approfondies) from Ecole Polytechnique Palaiseau (Paris, France); and a PhD from the University of Lausanne (Lausanne, Switzerland).

3. I have over _6_ years of experience relating to developing non-viral vector systems for gene and drug delivery, including _6_ years at the Imperial College Genetic Therapies Centre and _4_ years at IC-Vec, Ltd.
4. I have read and understand U.S. Patent Application Serial No. 10/770,294 (hereinafter "the '294 application"). It is my understanding that the '294 application has a U.S. filing date of February 2, 2004. The '294 application discloses using cationic liposomes with a head group that is more positive than the head group of DC-Chol to treat genetic disorders, conditions, or diseases. It is my understanding that the claims in the '294 application are generally directed to methods for treating a genetic disorder, condition, or disease by administering such liposomes. The '294 application discloses a variety of cationic liposomes containing DC-Chol polyamine analogues such as, for example, N'-cholesteryloxy-carbonyl-3,7,diaza-1,9-nonanediamine (CDAN).
5. The '294 application discloses delivering genes to cells via liposome/plasmid DNA complexes using, for example, one or more liposomes described in the '294 application. At pages 24-25, the '294 application discloses preparing cationic liposome/plasmid DNA complexes using either a pCF1-βGal plasmid expressing β-galactosidase or a pCF1-CAT plasmid expressing chloramphenicol acetyl transferase (CAT). Pages 18-19 and 24-25 disclose that *in vitro* gene delivery assays were performed with immortalized cystic fibrosis airway epithelial (CFT1) cells and *in vivo* studies were performed by intranasally administering the cationic liposome/plasmid DNA complexes into the lungs of female BALB/c mice. Figure 25 shows results obtained from testing gene delivery *in vivo* as measured by CAT expression.
6. It is known in the art and was known at the time the '294 application was filed that reporter gene assays may be and are often used to determine transfection ability of a vector. It is and was also known at the time the '294 application was filed that such reporter gene assays may be used to assess therapeutic potential. As an example, it is and was known in the art to evaluate therapeutic potential by measuring CAT expression. Consequently,

the '294 application's *in vivo* test results may also be considered to show therapeutic potential using liposomes in accordance with the disclosure in the '294 application.

7. I conducted *in vivo* experiments analyzing the transfection ability of a liposome disclosed in the '294 application. The experimental procedure and data for these tests are presented in Exhibit A accompanying this declaration. In particular, I prepared a CDAN:DOPE liposome having a CDAN:DOPE molar ratio of 66:29. This is about a ratio of 2:1, which the '294 application discloses as one of the molar ratios employed for the *in vitro* and *in vivo* tests. ('294 application, page 24, lines 22-27). I then prepared lipid/plasmid DNA lipoplexes with the CDAN:DOPE liposomes and a pDNA expressing the CAT reporter gene. The lipoplexes were prepared using dfferent liposome:nucleic pDNA (LD) ratios. The samples were instilled to female Balb/C mice (as disclosed in the '294 application), the animals sacrificed after 24 hours, the lungs extracted, homogenized, and measured for CAT activity. The transfection ability of these liposomes was compared to the transfection ability of the polymer polyethylenemine, which is known to give high transfection levels but also induces severe toxicity related to lung inflammation. As shown in Exhibit A, the liposomes provide good tranfection over a range of lipid:nucleic acid ratios. At some ratios, the transfection is equivalent to that obtained with polyethyleneimine but is not accompanied by the toxicity problems associated with polethyleneimine.
8. Based on my education, experience, and the tests I conducted in Exhibit A in this declaration, I declare that the '294 application enables a person skilled in the art to practice the methods claimed in that application. The '294 application discloses *in vivo* delivery of liposome/nucleic acid complexes using a CAT expressing plasmid. As discussed above, it is/was known in the art to analyze CAT expression to assess therapeutic potential. Given the *in vivo* testing disclosed in the '294 application that analyzes CAT expression and the knowledge in the art to use CAT expression to assess therapeutic potential, undue experimentation would not be required to evaluate the ability

of liposomes to treat a disease condition, or disorder as disclosed and claimed in accordance with the '294 application. Further, the experiments I conducted using CDAN:DOPE demonstrate that a person skilled in the art could, upon reading the disclosure in the '294 application, practice the claimed invention without undue experimentation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application and any patent issued thereon.

Michael Keller

Dr. Michael Keller

March 29, 2006__
Date

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